Aquaculture Studies, 25(5), 266-277, AQUAST2087
http://doi.org/10.4194/AQUAST2087

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RESEARCH PAPER

Amino Acid and Fatty Acid Profiles of Microalgae Cultivated in Aquaponics Remineralized Sludge Water

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How to Cite

Yeşiltaş, M., Koru, E. (2025). Amino Acid and Fatty Acid Profiles of Microalgae Cultivated in Aquaponics Remineralized Sludge Water. *Aquaculture Studies*, *25(5), AQUAST2087*. http://doi.org/10.4194/AQUAST2087

Article History
Received 27 August 2024
Accepted 28 May 2025
First Online 04 June 2025

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Keywords
Chlorella minutissima
Botryococcus braunii
Haematococcus pluvialis
Nutritional quality

Abstract

In this study, *Chlorella minutissima, Botryococcus braunii,* and *Haematococcus pluvialis* were selected to culture in Blue-Green 11 (BG-11) media, remineralized sludge water (RSW), and RSW including trace element solution (RSW+Mn) for 28 days. Post-harvest, amino acid and fatty acid profiles were determined. The total amino acid (ΣAA) content was observed in *C. minutissima*, measuring 68.62±6.35 g/100g, when cultivated in BG-11 culture media. The highest total fatty acid content was found in *B. braunii*, calculating 95.19±1.38 g/100g, when cultivated in BG-11 culture media. The total content of monounsaturated fatty acids was high compared to polyunsaturated fatty acids (PUFAs) in all groups. This study demonstrates the potential of *C. minutissima, B. braunii,* and *H. pluvialis* as a valuable source of essential amino and fatty acids. Among the 171 amino acid comparisons across different culture media, only five (aspartic acid, alanine, cysteine, phenylalanine, and isoleucine in *Botryococcus braunii*) exhibited statistically significant differences ($P<0.05$), suggesting that the overall amino acid composition remained relatively stable despite changes in medium formulation. No statistically significant differences were observed among the microalgae species in terms of their fatty acid profiles.

Introduction

Microalgae are a readily accessible, inexpensive, and sustainable resource with potential applications in diverse sectors, including food, feed, pharmaceuticals, cosmetics, and energy (Udayan et al., 2021; Show P. L., 2022). In recent years, research into the sustainable production of microalgae has intensified, driven by the need for eco-friendly and cost-effective cultivation methods (Samoraj et al., 2024). One promising approach involves the use of RSW from aquaponics, which contains high concentrations of nutrients essential for microalgae growth, offering a sustainable alternative to traditional nutrient sources. The resulting microalgae biomass can be further utilized as a high-quality fish feed resource, creating a circular and integrated system that enhances resource efficiency in aquaponics.

High-protein fish feeds, rich in nitrogen and phosphorus, are widely used in the aquaculture sector; however, less than 50% of these nutrients are utilized by the fish, leaving the remainder as potential pollutants within aquaculture systems (Piedrahita, 2003). Fish feed is a double-edged sword for aquaponic systems. While it provides essential nutrients for fish and plants, uneaten or undigested feed can become a major source of pollution.

Typical aquaponic systems that lack fecal solids mineralization see fish excrete varying amounts of soluble nutrients. Nitrogen excretion ranges from 36.9 to 44.0 g/kg feed, while phosphorus excretion falls

between 1.4 and 3.7 g/kg feed (Colt and Schuur, 2021). Elevated feeding rates can lead to increased concentrations of non-ionized ammonia, a potential toxin for cultured species. Conversely, carbon dioxide, ammoniacal nitrogen, and phosphorus act as essential plant nutrients, stimulating phytoplankton growth in aquaculture systems (Boyd and McNevin, 2022).

Bioremediation represents a well-established technology that utilizes beneficial microorganisms for the treatment of wastewater. Anaerobic digestion and aerobic treatment methods can be evaluated as established methodologies for nutrient conversion within aquaculture based sludge water (Xia et al., 2022). In addition to improving the quality of wastewater after aerobic and anaerobic remineralization, phycoremediation can play an important role in converting nutrient salts into biomass (Razaviarani et al., 2023). Phycoremediation encompasses the application of phototrophic or heterotrophic microalgae, macroalgae, and even cyanobacteria for pollutant removal or resource recovery from wastewater streams, including the generation of algal biomass. Phycoremediation's effectiveness and low cost in wastewater treatment make it a promising option for post-aerobic oxidation treatment.

Several studies have shown that phosphorus may influence the prebiotic formation of small organic molecules (amino acids, nucleosides, and basic building blocks) and larger, complex molecules (peptides and nucleotides) (Kolodiazhnyi, 2021). Nitrogen limitation within the culture media has been shown to potentially decrease biomass yield. Xu et al. (2001) observed this phenomenon in *Ellipsoidion* sp., where a decline in pH within an ammonium-supplemented culture media coincided with reduced growth rates but a concurrent increase in the accumulation of eicosapentaenoic acid (EPA) and polyunsaturated fatty acids (PUFAs).

In this study, *Chlorella minutissima, Botryococcus braunii* and *Haematococcus pluvialis* were used. All three species are studied for their ability to utilize nutrients (e.g., nitrogen, phosphorus) from wastewater or aquaculture effluents, making them valuable for nutrient recycling and bioremediation (Malla et al., 2015; Mkpuma et al., 2023; de Moraes et al., 2024). *Chlorella minutissima* is known for its rapid growth and high protein content, making it a valuable source of essential amino acids. *C. Minutissima* can used as a live feed or feed supplement for fish and shellfish due to its high protein content (Siddik et al., 2024). *Botryococcus braunii* is renowned for its ability to synthesize and accumulate large amounts of hydrocarbons and fatty acids, particularly lipids with biofuel potential. *B. braunii* is less commonly used in aquaculture but can contribute to nutrient recycling in integrated systems (Mkpuma et al., 2023). *Haematococcus pluvialis* is distinguished by its capacity to produce astaxanthin, a potent antioxidant, alongside its rich fatty acid profile. *H. Pluvialis* is used to enhance the pigmentation, survival, disease resistance, and health of farmed fish through its astaxanthin content (Yusoff et al., 2020). Evaluating the effects of reducing native biological contaminants and dissolved nutrients in aquaponics wastewater prior to inoculation could be essential for preventing biomass loss and maintaining the amino acid and fatty acid profiles of three different microalgae species cultivated under such conditions. To the best of our knowledge, this study to provide a detailed assessment of the nutritional quality of these microalgae species cultivated in aquaponics-based media, offering valuable insights into their nutritional potential and biotechnological applicability. This study aimed to characterize the amino acids and fatty acids concentration profiles of *C. minutissima, B. braunii*, and *H. pluvialis* cultivated in three media: BG-11, RSW, and RSW+Mn.

Material and Method

Microalgae Cultivation

Chlorella minutissima, Botryococcus braunii, and *Haematococcus pluvialis* was obtained from the General Directorate of Agricultural Research and Policies, Mediterranean Fisheries Research Production and Training Institute, Microalgae Culture Unit Antalya/Türkiye. These microalgae species were pre-cultured in BG-11 media (Rippka, 1992) at 25±2 °C under 36-watt illuminated fluorescent lamps with a 16:8 light: dark photoperiod and an aeration rate of 2.4 $Lmin^{-1}$. The ionic composition of the culture media of BG-11, RSW, and RSW+Mn presented in Table 1.

A total of 10 $Lday^{-1}$ of solid waste was collected from the effluent of the fish tank and separation tanks over a 42-day period (Yeşiltaş et al., 2023). Ten liters of each solid waste sample consisted of wastewater aerobically oxidized from the aquaponic system using an aeration rate of 5 $Lmin^{-1}$, daily. The aerated solid waste samples and prepared BG-11 media were subsequently autoclaved, put together in a water tank, filtered using glass microfiber filters (Whatman GF/F, 47 mm diameter, 0.7 µm pore size), and stored under sterile conditions. The microalgae cultivation experiment was initiated for a duration of 28 days in all culture media. Precultured microalgae were inoculated into 10-liter polyethylene plastic bags including different culture media, BG-11, RSW and RSW+Mn.

Temperature (°C), pH, and electrical conductivity (EC, μScm^{-1}) were monitored using a multiparameter instrument (YSI Pro ODO, Yellow Spring Instruments, Ohio, USA). At the end of the culture experiment, 500 mL from each trial was centrifuged at 6000 rpm for 10 minutes at 25 °C to harvest sufficient microalgal biomass (Mikro 22R, Hettich Zentrifugen, Tuttlingen, Germany) and subsequently frozen for storage. Microalgae biomass was measured at the beginning and end of the experiment using gravimetric methods with an analytical balance (Shimadzu ATX 224R, Kyoto, Japan) to calculate specific growth rates, biomass productivity, and doubling times. The frozen biomass was lyophilized

using a freeze dryer (Telstar Cryodos-50, Terrassa, Spain) for two days at 0.023 mBar and -55 °C to obtain dry biomass. All amino acid and fatty acid analyses were conducted on freze-dried microalgae biomass stored at -18 °C. Specific growth rate (μ, $hour^{-1}$) was determined from exponential growth phase using regression analysis $[\ln(X/X_0)/t]$, where X_0 and X were biomass dry weight concentrations (mgL^{-1}) at the initial time and the day n, respectively. t represents experiment time. Microalgae biomass productivity (P), as $mgL^{-1}day^{-1}$, was estimated with the formula: $P= (X_1-X_0)/(t_x-t_0)$, where X_0 and X were biomass dry weight concentrations (mgL^{-1}) at the initial day and the n days later, respectively. t represents experiment time. Microalgae doubling time (generation time) was calculated with following formula: $t_d = \ln(2)/\mu$.

Amino Acid Analysis

To determine the amount of amino acids, a Shimadzu Nexera-I LC-2040C 3D model high-performance liquid chromatography device (HPLC) and Sigma Aldrich AAS18 amino acid standard were used. For protein extraction, microalgae samples weighing 125 to 200 mg, adjusted based on protein ratios, were placed in a 6M HCl-phenol solution and heated in a thermoreactor at 120 °C for 24 hours (Cunniff and Washington, 1997). Subsequently, the samples were dried at 60 °C to remove water in the thermoreactor. The amino acids dissolved in 20 mL of pure water were then filtered through PTFE syringe filters with a pore diameter of 0.2 μm and analyzed using the HPLC device. Amino acid profiles were extracted following the manufacturer's instructions. High-performance liquid chromatography (HPLC) analyses were performed using a Shimadzu Nexera-i series LC-2040C 3D liquid chromatograph (Schimadzu, Kyoto, Japan) equipped with a column and sample thermostat, a degasser, an autosampler, and an ultraviolet detector. Amino acid content was expressed as grams of amino acid per 100 grams of protein.

Fatty Acid Analysis

Lipid extraction was conducted using the method described by Bligh and Dyer (1959). The fatty acid composition was determined using standards prepared by the American Oil Chemists' Society (AOCS, 1995). Methyl esters of fatty acids were prepared by transmethylation using 2 M KOH in n-hexane and methanol according to the method prepared by Ichihara et al. (1996). After the homogenization and centrifugation processes, the samples, which upper phase was removed, were taken into Eppendorf tubes and treated with nitrogen gas to remove chloroform (Jakobsen et al., 2008). Fatty acid analysis was performed using a Thermo Scientific Focus GC gas chromatograph (Thermo Fisher Scientific, Bremen, Germany) equipped with a flame ionization detector (FID) and a silica capillary column (60 m length, 0.25 mm diameter, and 0.00025 mm film thickness). The analysis employed a 5 μL sample injection volume with a carrier gas pressure of 16 psi. The derivatization reagent was utilized in a 1:40 ratio (sample: reagent) for fatty acid methylation. Identification of fatty acids was achieved by comparing their retention times with those of a known FAME (Fatty Acid Methyl Ester) standard mixture (SUPELCO). The total fatty acid concentration was determined by calculating the sum of the quantified saturated and unsaturated fatty acids (C12-C20) (Zhang et al., 2012). Fatty acid composition was expressed as a percentage of total fatty acid content.

Statistical Analysis

JMP Pro 13 software (SAS Institute Inc.) was employed for statistical analyses. The normality of the data distribution was assessed using the Shapiro–Wilk test. For each microalgal species, the effects of different culture conditions on amino acid and fatty acid profiles were evaluated using the non-parametric ANOVA (Kruskal–Wallis H test), followed by Dunn's post hoc comparison. Statistical significance was defined at $P<0.05$. Results are presented in tables as means±standard deviation.

Results and Discussion

C. minutissima, B. braunii, and *H. pluvialis* are recognized for their abundant amino acid and fatty acid profiles (Mehariya et al., 2021). Despite notable differences in the ionic composition among BG-11, RSW, and RSW+Mn culture media, only a limited number of amino acids in *Botryococcus braunii* exhibited statistically significant variations across the culture conditions. A strong correlation exists between nitrogen availability in microalgal culture media and the subsequent accumulation of biochemical constituents, such as proteins and lipids (Baroni et al., 2020; Udayan et al., 2023).

Microaalgae Cultivation

The growth performance of *Chlorella minutissima*, *Botryococcus braunii*, and *Haematococcus pluvialis* was assessed under different culture conditions, including BG-11, RSW), and RSW+Mn. The temperature remained relatively stable across all treatments, ranging from 24.32°C to 26.76°C, with *B. braunii* exhibiting slightly higher temperatures compared to the other species. The pH varied between 7.84 and 8.64, with the highest values observed in *B. braunii* cultures. EC was significantly higher in *H. pluvialis* cultured in RSW+Mn (2215±59 μScm^{-1}) compared to other treatments, whereas the lowest EC was recorded in *B. braunii* in RSW+Mn (1155±43 μScm^{-1}). *C. minutissima*, *B. braunii*, and *H. pluvialis* reached their maximum cell densities of $2.7\times10^{-7}\pm1.17\times10^{-7}$ $cellsmL^{-1}$ on the 27th day in RSW+Mn

Table 1. The ionic composition of BG-11, RSW, and RSW+Mn

Descriptions	BG-11	RSW	RSW+Mn
NO_2-N (mg/L)	-	3.97±6.83	3.97±6.83
NO_3-N (mg/L)	247.06	4.32±7.9	4.32±7.9
PO_4-P (mg/L)	9.25	1.52±1.65	1.52±1.65
SO_4 (mg/L)	29.33	109.42±43.41	109.42±43.41
NH_4-N (mg/L)	0.32	1.06±1.18	1.06±1.18
K (mg/L)	5.67	8.68±5.15	8.68±5.15
Mg (mg/L)	7.40	33.36±7.88	33.36±7.88
Ca (mg/L)	9.81	155.5±29.36	155.5±29.36
Na (mg/L)	410.29	55.65±58.14	55.65±58.14
Cl (mg/L)	9.00	89.87±132.74	89.87±132.74
Co (mg/L)	0.0091	-	0.0091
Mo (mg/L)	0.1546	-	0.1546
Mn (mg/L)	0.5025	-	0.5025
Zn (mg/L)	0.0500	-	0.0500
Cu (mg/L)	0.0204	-	0.0204

BG-11: Blue-green microalgae culture media, RSW: Remineralized sludge-water, RSW+Tr: Remineralized sludge-water+BG-11 microalgae culture media trace element solution.

media, $3.6\times10^{-5}\pm4.7\times10^{-4}$ cellsmL^{-1} on the 21st day in RSW+Mn media, and $8.9\times10^{-4}\pm1.9\times10^{-4}$ cellsmL^{-1} on the 20th day in RSW+Mn media, respectively.

The specific growth rate (μ) was observed in *C. minutissima* cultured in BG-11 (0.100±0.013 day^{-1}), while *H. pluvialis* showed the lowest growth rates across all conditions. Biomass productivity (P) varied significantly among species and treatments, with *B. braunii* in RSW exhibiting the highest productivity (44.05±6.23 mgL^{-1}day^{-1}), whereas the lowest was recorded in *H. pluvialis* cultured in BG-11 (3.00±0.13 mgL^{-1}day^{-1}). The doubling time (t_d) was shortest in *C. minutissima* cultured in BG-11 (7.05±0.98 hours) and longest in *H. pluvialis* in the same medium (17.40±5.69 hours). These results indicate that culture medium composition plays a crucial role in regulating microalgal growth, with RSW+Mn supplementation improving productivity in *H. pluvialis* but not in *C. minutissima* and *B. braunii* (Table 2).

Values in the table are averages of three replicates±standard deviations. Temperature (°C), electrical conductivity (EC, μScm^{-1}), specific growth rate (μ, day^{-1}), biomass productivity (*P,* mg L^{-1}day^{-1}), and doubling time (t_d, hour) are presented.

Amino Acids

Amino acid profiles of *C. minutissima*, *B. braunii*, and *H. pluvialis* cultivated in BG-11, RSW, and RSW+Mn were analysed. Nutrient composition significantly influences intracellular nitrogen assimilation and protein metabolism (Song et al.,2024). Results showed that 17 amino acids (AAs) were found in *C. minutissima, B. braunii*, and *H. pluvialis*. 8 are essential amino acids (eAAs) i.e., histidine (His), threonine, valine, phenylalanine, isoleucine, leucine, lysine, and tryptophan (Trp). Tyrosine (Tyr) was the most abundant AAs amongst all microalgae species and treatments as shown in Table 3 and Figure 1. Among individual amino acids, Aspartic acid (Asp), Glutamine (Glu), and Tyrosine (Tyr) were found to be consistently dominant across all species and media. Histidine and Tryptophan consistently exhibited the lowest concentrations among all amino acids across both microalgal species and culture media (Table 3 and Figure 1). *C. minutissima* cultures grown in RSW media exhibited the highest total amino acid content. Conversely, the lowest amino acid content was observed in *C. minutissima* cultured in RSW+Mn media. The total amino acid (ΣAA) content of all microalgae exhibited variation across culture media. *C. minutissima* cultures grown in BG-11 media exhibited the highest total amino acid content (68.62±6.35 g/100g), followed by RSW+Mn media (59.79±3.50 g/100g), suggesting a degree of metabolic resilience in this species. Notably, *C. minutissima* accumulated exceptionally high Tyr levels in BG-11 (12.02±1.99 g/100g), suggesting elevated flux through the shikimate pathway under favorable nutrient conditions (Hermann and Weaver, 1999). This suggests differential regulation of branched-chain amino acid synthesis, which may be linked to variations in carbon: nitrogen balance or trace element availability, particularly in the RSW-based treatments. *H. pluvialis* cultured in RSW+Mn media exhibited the lowest total amino acid content, averaging approximately 40.02±3.96 g/100g, likely reflecting nutrient stress or insufficient nitrogen uptake under this condition. The total essential amino acid (ΣEAA) content was notably influenced by culture conditions in *H. pluvialis*, where the BG-11 medium supported significantly higher levels (19.11±1.27 g/100g) compared to RSW+Mn (13.87±1.79 g/100g). This is particularly relevant for biotechnological applications where essential amino acid enrichment is desired, such as in aquafeed formulation or nutraceuticals. Although RSW-based media are attractive from a sustainability perspective, our findings suggest that without careful supplementation—especially with nitrogen and trace elements—they may compromise biochemical quality. Nevertheless, the comparable performance of *B. braunii* and *C. minutissima* across media types highlights the potential for strain-specific adaptation to non-conventional nutrient sources. Analysis of the total

Table 2. Microalgae culture conditions and growth parameters

	C. minutissima			*B. braunii*			*H. pluvialis*		
Parameters/ Application	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn
Temperature	24.59±1.23^{a}	24.32±1.26^{a}	24.42±1.26^{a}	26.70±1.67^{a}	26.47±1.55^{a}	26.76±1.54^{a}	24.34±1.27^{a}	24.43±1.24^{a}	24.60±1.25^{a}
pH	8.06±0.25^{a}	8.42±0.41^{a}	8.39±0.36^{a}	8.05±0.34^{a}	8.64±0.19^{a}	8.51±0.15^{a}	7.84±0.15^{a}	8.27±0.34^{a}	8.23±0.32^{a}
EC	1559±247^{a}	990±26^{a}	975±16^{a}	1327±44^{a}	1184±61^{a}	1155±43^{a}	1604±103^{a}	1151±38^{a}	2215±59^{a}
μ	0.100±0.013^{a}	0.070±0.010^{a}	0.068±0.016^{a}	0.061±0.012^{a}	0.086±0.005^{a}	0.053±0.013^{a}	0.044±0.012^{a}	0.079±0.008^{a}	0.080±0.015^{a}
P	5.15±1.24^{a}	3.42±1.05^{a}	4.12±0.51^{a}	32.6±23.15^{a}	44.05±6.23^{a}	13.46±1.50^{a}	3.00±0.13^{a}	10.40±5.75^{a}	45.49±17.10^{a}
t_d	7.05±0.98^{a}	10.18±1.67^{a}	10.96±3.04^{a}	11.78±2.58^{a}	8.04±0.47^{a}	13.31±0.80^{a}	17.40±5.69^{a}	8.83±0.93^{a}	8.92±1.45^{a}

Table 3. Amino acid composition (DW of total protein (g/100g)) in *Chlorella minutissima*, *Botryococcus braunii*, and *Haematococcus pluvialis* in different culture media

Amino acids	*C. minutissima*			*B. braunii*			*H. pluvialis*		
	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn
Asp	7.49±0.39^{a}	5.53±1.14^{a}	8.12±0.38^{a}	7.90±0.91^{x}	6.01±0.28^{xy}	5.42±0.30^{y}	7.89±0.08^{k}	5.83±0.59^{k}	5.12±0.66^{k}
Glu	6.93±0.47^{a}	5.49±1.14^{a}	6.37±0.29^{a}	6.07±0.46^{x}	6.54±0.22^{x}	5.82±0.27^{x}	6.30±0.21^{k}	5.84±0.90^{k}	5.01±0.18^{k}
Ser	3.24±0.02^{a}	2.50±0.57^{a}	2.80±0.22^{a}	2.75±0.29^{x}	2.97±0.09^{x}	2.59±0.28^{x}	2.74±0.34^{k}	2.15±0.09^{k}	1.93±0.12^{k}
His	0.75±0.24^{a}	0.44±0.20^{a}	0.43±0.16^{a}	0.38±0.17^{x}	0.76±0.07^{x}	0.72±0.10^{x}	0.25±0.12^{k}	0.48±0.14^{k}	0.31±0.23^{k}
Gly	4.34±0.52^{a}	3.34±0.76^{a}	3.60±0.17^{a}	3.54±0.33^{x}	3.61±0.09^{x}	3.06±0.27^{x}	3.48±0.33^{k}	3.27±0.46^{k}	2.56±0.05^{k}
Thr	4.54±0.33^{a}	3.65±0.94^{a}	3.46±0.49^{a}	3.73±0.35^{x}	4.33±0.25^{x}	3.96±0.36^{x}	3.62±0.25^{k}	3.44±0.40^{k}	2.66±0.31^{k}
Ala	2.41±0.40^{a}	2.31±0.70^{a}	3.08±0.08^{a}	3.13±0.30^{x}	2.66±0.17^{xy}	1.85±0.08^{y}	2.33±0.15^{k}	1.79±0.12^{k}	1.30±0.31^{k}
Tyr	12.02±1.99^{a}	9.78±2.49^{a}	9.90±0.55^{a}	9.16±0.91^{x}	9.96±0.08^{x}	8.15±0.65^{x}	8.18±0.29^{k}	9.35±2.58^{k}	6.32±1.14^{k}
Cys	1.63±0.16^{a}	0.87±0.46^{a}	1.71±0.17^{a}	1.67±0.26^{x}	1.26±0.07^{xy}	0.89±0.17^{y}	1.67±0.11^{k}	0.97±0.14^{k}	0.62±0.30^{k}
Val	3.85±0.42^{a}	3.02±0.65^{a}	3.08±0.08^{a}	2.93±0.21^{x}	3.37±0.10^{x}	2.72±0.39^{x}	2.66±0.03^{k}	2.97±0.59^{k}	2.10±0.10^{k}
Trp	0.96±0.14^{a}	0.51±0.16^{a}	0.70±0.15^{a}	0.52±0.04^{x}	0.46±0.08^{x}	0.46±0.11^{x}	0.34±0.06^{k}	0.67±0.04^{k}	0.30±0.19^{k}
Phe	3.33±0.40^{a}	2.47±0.58^{a}	2.41±0.27^{a}	2.29±0.08^{x}	2.77±0.08^{y}	2.47±0.11^{xy}	2.22±0.27^{k}	2.55±0.35^{k}	1.77±0.06^{k}
Ile	2.38±0.17^{a}	1.85±0.44^{a}	2.97±0.19^{a}	2.96±0.35^{x}	2.27±0.13^{xy}	1.97±0.20^{y}	3.10±0.20^{k}	2.11±0.10^{k}	1.81±0.30^{k}
Leu	5.51±0.70^{a}	4.32±1.19^{a}	4.99±0.26^{a}	4,68±0.43^{x}	4.98±0.19^{x}	4.23±0.39^{x}	4.53±0.20^{k}	4.36±0.86^{k}	3.11±0.32^{k}
Lys	3.92±0.38^{a}	2.72±0.82^{a}	2.64±0.17^{a}	2.46±0.29^{x}	3.23±0.11^{x}	2.53±0.23^{x}	2.39±0.15^{k}	2.70±0.43^{k}	1.80±0.28^{k}
Hyp	2.35±0.22^{a}	1.84±0.41^{a}	1.63±0.17^{a}	1.75±0.16^{x}	2.19±0.02^{x}	2.09±0.26^{x}	1.57±0.10^{k}	2.32±0.43^{k}	1.34±0.14^{k}
Pro	2.96±1.22^{a}	2.29±1.02^{a}	1.89±0.40^{a}	1.79±0.69^{x}	3.39±0.18^{x}	2.25±0.23^{x}	1.34±0.69^{k}	1.56±0.10^{k}	1.94±0.48^{k}
ΣEAA	25.24±2.77^{a}	18.98±4.99^{a}	20.68±1.77^{a}	19.96±1.92^{x}	22.17±1.01^{x}	19.06±1.88^{x}	19.11±1.27^{k}	19.27±2.89^{k}	13.87±1.79^{k}
Total	68.62±6.35^{a}	53.30±13.33^{a}	59.79±3.50^{a}	57.70±5.95^{x}	60.75±1.52^{x}	51.18±4.06^{x}	54.59±1.88^{k}	52.35±8.03^{k}	40.02±3.96^{k}

Values in the table represent the mean ± standard deviation of three replicates. The statistical analysis was performed with a confidence interval of 0.05. Different letters indicate statistically significant differences between groups (Kruskal-Wallis nonparametric test, Dunn's post-hoc comparison test, P<0.05).

amino acid content revealed that the *B. braunii* species cultured in RSW media exhibited significantly higher yields compared to other culture media. Minimal variation in total amino acid content was observed between the *H. pluvialis* strain cultured in BG-11 (≈60 g/100g) and RSW media (≈59 g/100g). The total amino acid content of *H. pluvialis* cultivated in RSW+Mn media was approximately 49 g/100g. Total amino acid content exhibited a slight increase in *C. minutissima* and *H. pluvialis* cultured in BG-11. In contrast, *B. braunii* cultivated in RSW media demonstrated higher amino acid accumulation compared to other culture conditions. These results support previous findings that the growth medium composition exerts a direct impact on nitrogen metabolism and protein synthesis pathways in microalgae (Lourenço et al., 2004; Zhu et al., 2021; Araya et al., 2021).

Individual amino acid profiles exhibited diverse patterns across the three algae species under the BG-11, RSW, and RSW+Mn culture conditions (Figure 1). Comparative analysis of amino acid concentrations among microalgae cultured in BG-11, RSW, and RSW+Mn media revealed statistically significant differences in only a small subset of amino acids, suggesting that variations in macro- and micronutrient composition had a minimal overall impact on amino acid profiles. A comparative analysis of *Chlorella sorokiniana* amino acid profiles between this study and that of Ribeiro et al. (2019) revealed discrepancies in the abundances of Ala, Glu, Ser, Val, and Thr. Previous studies have documented a 36 g/100g reduction in protein content during the palmella phase of *H. pluvialis* development. Additionally, protein analysis of the red stage indicated a predominance of Asp, Glu, Ala, and Leu. Despite the absence of a complete palmella phase in the *Haematococcus pluvialis* cultures, the observed trends were consistent with previous findings reported by Oslan et al. (2021). Total amino acid concentrations of *B. braunii* in this study surpassed those reported by Araya et al. (2021) across all experimental groups. Total essential amino acid (ΣeAA) concentrations in microalgae cultured under varying media conditions were significantly elevated compared to previous studies (Liang et al., 2020, Nateghpour et al., 2021). The results highlight the dominant role of species-specific factors in determining amino acid content, with species accounting for over 90% of the observed variation. This suggests that metabolic pathways, genetic factors, and other intrinsic biological characteristics are the primary drivers of amino acid profiles in microalgae. While culture conditions also had a significant effect, their impact was relatively minor, accounting for less than 0.5% of the total variation.

The significant interaction effect indicates that the response of each species to different culture conditions is not uniform. For example, some species may show

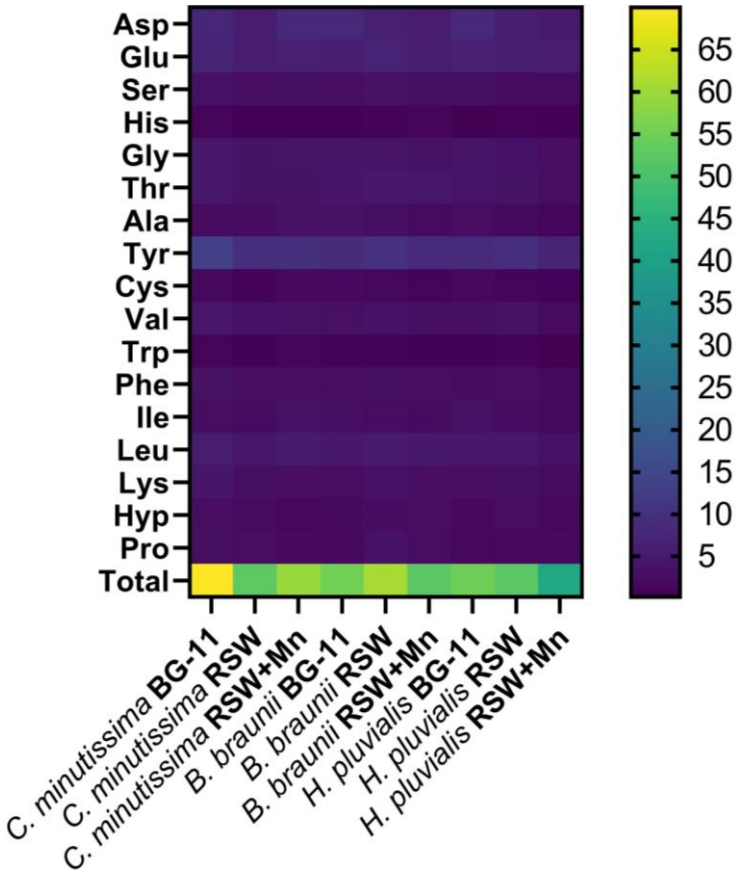

Figure 1. Amino acid concentrations of *Chlorella minutissima*, *Botryococcus braunii*, and *Haematococcus pluvialis* cultivated in BG-11, RSW, and RSW+Mn media.

increased amino acid content in BG-11, while others may respond better to RSW or RSW+Mn. These findings underscore the importance of tailoring culture conditions to specific species to optimize amino acid production. Further studies could explore the underlying mechanisms driving these species-specific responses and investigate additional culture conditions to enhance amino acid yields.

Values in the table represent the mean ± standard deviation of three replicates. The statistical analysis was performed with a confidence interval of 0.05. Different letters indicate statistically significant differences between groups (Kruskal-Wallis nonparametric test, Dunn's post-hoc comparison test, $P<0.05$).

The data also point to distinct species-specific strategies for managing amino acid metabolism under varying environmental conditions. For instance, *C. minutissima* appears to maintain a relatively stable amino acid profile regardless of medium, potentially due to robust homeostatic control mechanisms. In contrast, *H. pluvialis* shows a more dynamic and stress-responsive profile, consistent with its known sensitivity to nutrient limitations and tendency to divert resources toward secondary metabolite production under stress (e.g., carotenoids like astaxanthin). These biochemical patterns underline the importance of aligning culture conditions with the intended application—whether maximizing protein content, amino acid balance, or co-product yield.

Fatty Acids

The health-promoting properties of fatty acids derived from microalgae species are well-established in the scientific literature (Liu et al., 2022). Fatty acid profiles of *C. minutissima*, *B. braunii*, and *H. pluvialis* cultivated in BG-11, RSW, and RSW+Mn were analysed and varied markedly across culture media, reflecting species-specific lipid biosynthesis responses to nutrient availability and environmental conditions. The fatty acids of all microalgae based on the total FAs are shown in Table 4 and Figure 2. A standard mixture containing 14 fatty acids (FAs) was employed for fatty acid profile analysis of the microalgae. Across all species, monounsaturated fatty acids (MUFAs) were the predominant class, with *C. minutissima* in RSW+Mn exhibiting the highest MUFA content (86.64±4.85%). This observation aligns with previous studies indicating that moderate stress or nutrient fluctuation often promotes MUFA accumulation, possibly due to the redirection of carbon flux toward simpler, energy-efficient fatty acids under suboptimal growth conditions (Aremu et al., 2015). The maximum total fatty acid (TFA) content was determined as 95.90±1.35 g/100g in *B. braunii* cultivated in BG-11 culture media. The minimum TFA content was calculated as 81.53±0.36 g/100g in *H. pluvialis* cultivated in BG-11 culture media. Statistical analysis revealed no significant differences in saturated, monounsaturated, polyunsaturated fatty acid profiles, or TFA content among all microalgae species cultured in BG-11, RSW, and RSW+Mn media (Table 4).

Among individual fatty acids, C17:1 (heptadecenoic acid) emerged as a major MUFA component in all three species, particularly in *C. minutissima* and *B. braunii*, where levels exceeded 40–68% of total fatty acids. This is noteworthy, as C17:1 is a relatively rare but industrially valuable fatty acid, linked to improved cold-flow properties in biofuels (Tibbetts et al., 2015). Its significant increase in *C. minutissima* under RSW+Mn suggests that trace element-enriched waste streams can stimulate specific desaturation pathways, offering an economical route to high-value lipid profiles. Interestingly, *H. pluvialis* maintained more consistent C17:1 levels across media, possibly indicating a regulatory ceiling on this pathway in this strain.

Saturated fatty acid (SFA) profile also revealed important trends. *B. braunii* accumulated the highest SFAs (up to 9.55±0.55% in RSW), dominated by C16:0 and C20:0. These values were significantly higher ($P<0.05$) than in *H. pluvialis*, which exhibited the lowest SFA content overall. The PUFA/SFA ratio, often used as a nutritional and oxidative stability index, was particularly elevated in *H. pluvialis*, reaching values above 5.0 in BG-11, highlighting this strain's potential for applications where high PUFA content is desired, such as functional foods or aquafeeds. However, PUFAs in *C. minutissima* were more sensitive to media composition, showing a substantial drop in RSW+Mn (3.57±0.77%) compared to BG-11 and RSW, indicating possible downregulation of PUFA synthesis pathways under trace element-enriched conditions.

Fatty acid profiles exhibited distinct variations across microalgae species and culture conditions (Figure 2). There were 14 fatty acids detected in all groups except *B. braunii*. Stearidonic acid (C18:4n3) was very low in all *B. braunii* samples cultured in BG-11, RSW, and RSW+Mn media. The most dominant fatty acids in all microalgae groups were heptadecanoic acid (C17:1), cis-10-pentadecanoic acid methyl ester (C15:1), elaidic acid (C18:1n9), eicosenoic acid (C20:1), and gamma-linolenic acid (C18:3n6) (Table 4). Oleic acid (C18:1n9), a fatty acid associated with cardiovascular benefits (Saeed et al., 2025), exhibited variability among microalgae species and culture conditions. The highest C18:1n9 content was observed in *C. minutissima* cultured in RSW media. Gamma-linolenic acid (C18:3n6), a fatty acid critical for brain, skeletal, reproductive, and metabolic functions (Chauhan et al. 2024), was most abundant in *H. pluvialis* cultured in BG-11 media, reaching approximately 11% of total fatty acid content. Linoleic acid (C18:2ω6), α-linolenic acid (C18:3ω3), and γ-linolenic acid (C18:3ω6) are the predominant polyunsaturated fatty acids (PUFAs) in plants, primarily concentrated in seeds and nuts oil (Blasio and Balzano, 2021). Monounsaturated fatty acid content was notably elevated relative to literature data, whereas polyunsaturated fatty acid levels remained comparatively low across all groups (Kumaran et al.,

Table 4. Fatty acid content (%DW) in *Chlorella minutissima*, *Botryococcus braunii*, and *Haematococcus pluvialis* in different culture media

Fatty acids	*C. minutissima*			*B. braunii*			*H. pluvialis*		
	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn	BG-11	RSW	RSW+Mn
C13:0	0.17±0.04^{a}	0.43±0.15^{b}	0.23±0.00^{ab}	0.18±0.01^{x}	0.17±0.02^{x}	0.14±0.01^{x}	0.42±0.10^{k}	0.30±0.05^{k}	0.33±0.00^{k}
C16:0	0.72±0.12^{a}	0.63±0.19^{a}	0.32±0.02^{a}	4.53±0.38^{x}	4.02±0.02^{x}	3.86±0.48^{x}	0.44±0.14^{k}	0.38±0.01^{k}	0.41±0.01^{k}
C17:0	0.30±0.13^{a}	0.24±0.07^{a}	0.11±0.00^{a}	0.34±0.02^{x}	0.37±0.05^{x}	0.40±0.08^{x}	0.10±0.04^{k}	0.00±0.00^{k}	0.00±0.00^{k}
C18:0	0.49±0.19^{a}	0.75±0.21^{a}	0.67±0.43^{a}	0.26±0.01^{x}	0.24±0.05^{x}	0.28±0.04^{x}	0.64±0.11^{k}	0.56±0.07^{k}	0.59±0.04^{k}
C20:0	4.79±0.78^{ab}	7.16±0.40^{a}	1.03±0.48^{b}	2.02±0.30^{x}	2.39±0.33^{x}	0.97±0.35^{x}	1.61±0.04^{k}	1.93±0.18^{k}	2.11±0.09^{k}
C21:0	0.29±0.02^{a}	0.34±0.02^{a}	0.41±0.09^{a}	0.25±0.01^{x}	0.26±0.01^{x}	0.24±0.33^{x}	0.25±0.03^{k}	0.26±0.03^{k}	0.24±0.00^{k}
ΣSFA	6.70±1.11^{ab}	9.55±0.55^{a}	3.35±2.02^{b}	7.15±0.24^{x}	7.45±0.28^{x}	5.89±0.64^{x}	2.89±0.87^{k}	3.42±0.27^{k}	3.67±0.13^{k}
C15:1	12.39±1.07^{a}	13.84±0.67^{a}	8.87±1.31^{a}	14.8±0.92^{x}	13.98±0.31^{x}	15.58±1.31^{x}	12.89±1.87^{k}	10.31±0.19^{k}	10.56±0.43^{k}
C16:1	0.46±0.34^{a}	0.54±0.43^{a}	0.15±0.01^{a}	0.13±0.06^{x}	0.12±0.01^{x}	0.12±0.01^{x}	0.16±0.01^{k}	0.20±0.07^{k}	0.15±0.01^{k}
C17:1	43.06±0.34^{a}	40.38±5.75^{a}	68.00±10.16^{a}	55.4±1.39^{x}	52.22±0.90^{x}	56.01±4.38^{x}	40.79±8.03^{k}	52.56±6.08^{k}	49.39±1.26^{k}
C18:1n9	12.14±1.94^{a}	12.24±0.77^{a}	7.41±3.11^{a}	1.85±0.11^{x}	2.11±0.10^{x}	1.82±0.20^{x}	4.47±1.96^{k}	3.71±0.50^{k}	4.09±0.18^{k}
C20:1	8.41±2.51^{a}	7.91±1.38^{a}	2.22±0.98^{a}	7.51±0.89^{x}	4.97±0.21^{x}	5.66±1.06^{x}	7.15±1.24^{k}	4.99±0.82^{k}	5.50±0.12^{k}
ΣMUFA	76.46±6.39^{a}	74.91±3.01^{a}	86.64±4.85^{b}	79.7±0.53^{x}	73.40±0.88^{x}	79.19±2.20^{x}	65.46±3.53^{k}	71.76±4.65^{k}	69.68±0.53^{k}
C18:2n6	0.81±0.21^{a}	0.75±0.09^{a}	1.29±0.17^{a}	1.00±0.02^{x}	0.98±0.02^{x}	0.99±0.08^{x}	0.83±0.14^{k}	0.98±0.12^{k}	0.97±0.04^{k}
C18:3n6	4.77±1.30^{a}	5.29±0.83^{a}	1.77±0.67^{a}	7.32±0.72^{x}	8.09±0.52^{x}	6.86±1.45^{x}	10.90±2.42^{k}	7.67±1.52^{k}	8.85±0.28^{k}
C18:4n3	1.99±0.87^{a}	1.86±0.13^{a}	0.51±0.27^{a}	0.27±0.01^{x}	0.26±0.01^{x}	0.24±0.01^{x}	1.16±0.19^{k}	0.91±0.07^{k}	1.09±0.02^{k}
ΣPUFA	7.57±1.94^{a}	7.9±0.85^{a}	3.57±0.77^{a}	8.32±0.71^{x}	9.08±0.52^{x}	7.85±1.38^{x}	12.89±2.47^{k}	9.56±1.47^{k}	10.91±0.27^{k}
ΣFA	90.73±3.78^{a}	92.36±1.66^{a}	93.56±2.08^{a}	95.1±1.38^{x}	89.93±1.28^{y}	92.93±0.81^{xy}	81.24±1.99^{k}	84.73±2.92^{k}	84.25±0.39^{k}
PUFA/ SFA	1.15±0.27^{a}	0.82± 0.04^{a}	1.33±0.47^{a}	1.16±0.08^{x}	1.22±0.05^{x}	1.33±0.14^{x}	5.03±2.14^{k}	2.78±0.21^{k}	2.98±0.18^{k}
Other	9.27±3.78^{a}	7.64±1.66^{a}	6.44±2.08^{a}	4.81±1.38^{x}	10.07±1.28^{y}	7.07±0.81^{xy}	18.76±1.99^{k}	15.28±2.92^{k}	15.75±0.39^{k}

Values in the table represent the mean ± standard deviation of three replicates. The statistical analysis was performed with a confidence interval of 0.05. Different letters indicate statistically significant differences between groups (Kruskal-Wallis nonparametric test, Dunn's post-hoc comparison test, P<0.05).

2023). Total saturated fatty acid content in this study was lower than that reported previously for *Scenedesmus bijugus* cultured in BG-11 media (Minhas et al. 2020). These results support previous findings that the composition of the growth medium directly influences lipid metabolism and fatty acid biosynthesis pathways in microalgae (Tang et al., 2011; Kurt et al., 2022; Marinho et al., 2021).

Total fatty acid (ΣFA) content remained relatively stable across media for most species, except in *H. pluvialis*, where the lowest values were observed in RSW+Mn (84.25±0.39%). The "Other" lipid fraction—possibly representing unidentified or minor fatty acids—was highest in *H. pluvialis*, especially in BG-11, suggesting enhanced synthesis of structurally diverse or oxygenated lipid species, which are often associated with stress adaptation mechanisms or secondary metabolism (Hu et al., 2021). These lipids, though present in smaller quantities, can have disproportionate functional impacts and merit further characterization.

Values in the table represent the mean ± standard deviation of three replicates. The statistical analysis was performed with a confidence interval of 0.05. Different letters indicate statistically significant differences between groups (Kruskal-Wallis nonparametric test, Dunn's post-hoc comparison test, $P<0.05$).

Conclusions

Remineralized aquaponics-based sludge water yielded comparable amino acid and fatty acid profiles to those obtained using BG-11 culture media. Our results showed that there were comparable results in amino acid and fatty acid in *C. minutissima, B. braunii*, and *H. pluvialis* when grown at RSW media. These microalgae species exhibit significantly higher biomass accumulation rates compared to terrestrial plants, positioning them as potential alternatives to edible oils derived from palm, sunflower, and canola. Furthermore, the amino acid profile presents potential for the extraction of bioactive compounds, with particular application in animal feed or high quality fertilizer within the agricultural sector. From a biotechnological perspective, the observed variations in fatty acid classes and individual profiles emphasize the importance of species-specific media optimization. While RSW and RSW+Mn offer sustainable cultivation options, their impact on lipid profiles is nuanced: they can enhance certain valuable fatty acids (e.g., MUFAs like C17:1) but may compromise PUFA production, depending on the strain. The elevated concentrations of amino acids and fatty acids observed in various microalgae species cultivated in distinct culture media, as compared in this

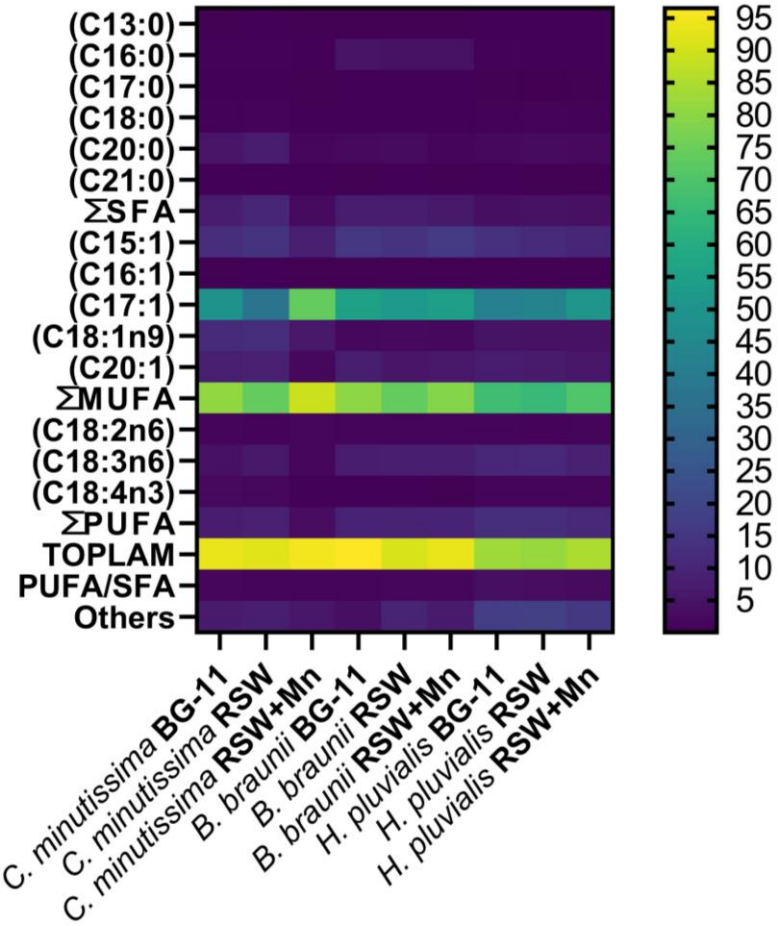

Figure 2. Fatty acid concentrations of *Chlorella minutissima*, *Botryococcus braunii*, and *Haematococcus pluvialis* cultivated in BG-11, RSW, and RSW+Mn media.

study, are of significant interest for industries seeking alternative sources of renewable and sustainable raw materials for the production of food supplements, pharmaceuticals, animal feed, energy, and cosmetics. This study demonstrates that microalgal fatty acid biosynthesis is highly responsive to environmental inputs, and that carefully selected nutrient regimes can steer lipid composition in desired directions. Further studies could explore additional variables, such as light intensity, temperature, nutrient availability, or even remineralization potential of wastewater from diverse sources to better understand the factors influencing amino acid and fatty acid production in microalgae.

Ethical Statement

All experimental process and protocols have been approved by Mediterranean Fisheries Research Production and Training Institute, Animal Experiments Local Ethics Committee (Report Number: 2019/01) under the General Directorate of Agricultural Research and Policy surveillance.

Funding Information

This research was financially supported by the Scientific and Technological Research Council of Türkiye (Grant number 119O180) and the Turkish Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies.

Author Contribution

Murat Yeşiltaş: Conceptualization, system setup, analyses, data evaluation and writing.

Edis Koru: Supervision, review, and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely thank Mr. Serkan Erkan, Manager of MEDFRI, for his support; Mr. Soner Sezen, Head of the Institute's Aquarium Unit, for his invaluable assistance and encouragement throughout the project; and Dr. Faruk Pak for his support with the fatty acid analysis. We are deeply thankful to the editors for their constructive feedback and to the reviewers for their insightful comments, which greatly enhanced the clarity and quality of the manuscript. This research was financially supported by the Scientific and Technological Research Council of Türkiye (Grant number 119O180) and the Turkish Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies.

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